**9-Arylimino noscapinoids as potent tubulin binding anticancer agent: chemical synthesis and cellular evaluation against breast tumor cells**

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**Chemical synthesis of the 9-Arylimino noscapinoids, 12-14**

**General**

All the reactions were monitored by TLC (Precoated silica plates and visualizing under UVlight). Reagents and all solvents were analytically pure and were used without further purification. Air-sensitive reagents were transferred by syringe or double-ended needle. Evaporation of solvents was performed at reduced pressure by using heidolph rotary evaporator.1H and 13C NMR spectra of samples in CDCl3were recorded on AVANCE-300MHz, 400 MHz, 500 MHz spectrometer. Chemical shift reported are relative to an internal standard TMS (δ=0.0). Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants are reported in hertz (Hz). Mass spectra were recorded in ESI conditions at 70 eV on LC-MSD (Agilent technologies) spectrometers. All high-resolution spectra were recorded on QSTAR XL hybrid MS/MS system (Applied Bio systems/ MDS sciex, foster city, USA), equipped with an ESI source (IICT, Hyderabad). Column chromatography was performed on silica gel (60-120 mesh) supplied by Acme Chemical Co., India. TLC was performed on Merck 60 F-254 silica gel plates. Commercially available anhydrous solvents Dichloromethane, methanol, acetone and Ethylacetate were used as such without further purification. Natural α-noscapine was purchased from Sigma-Aldrich.

***General procedure for chemical synthesis of 9-arylimino noscapinoids, 12-14***

The natural α-noscapine was used as a starting material to produced 9-aminonoscapine **6** via a two reaction steps involving bromination of noscapine using aqueous HBr/Br2-H2O followed by amination using CuI, NaN3 and L-Proline in DMSO as reported earlier [11]. A solution of 9-aminonoscapine **6** (1.0 mmol), in ethanol (15 mL), was refluxed with substituted aryl/heteroaryl aldehydes (2,5-difluorobenzaldehyde or 5-bromothiophenecarboxaldehyde or p-bromo benzaldehyde, 1.0 mmol), for 12 h. After the starting material was completely consumed in the reaction (judged by TLC), the solvent was evaporated under vacuum. The crude residue was extracted into dichloromethane (2 x 15 mL) and washed with brine solution. The organic layer was collected and passed through a Na2SO4 bed and later on removed under reduced pressure. The crude residue was chromatographed over a triethylamine silica bed, using pet.ether/ethyl acetate (7:3) as eluents, to produce 9-arylimino noscapinoids, **12-14** (Figure 4) as solid products in very good yield. Structural characterization of all the intermediates and final products **12-14** were done using NMR (1H and 13C), IR spectroscopy and mass (HRMS) spectrometry techniques.











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**Cellular proliferation study**

***Cell culture and reagents***

Noscapine was obtained from Sigma. All the chemical reagents and media for cell culture were obtained from Sigma. Human breast cancer cell line, MCF-7 and MDAMB-231 were obtained from the cell repository of the National Center for Cell Science Pune, Maharashtra, India. Primary breast cancer cells were isolated from the patient’s samples. Stock solution (100 mM) of the newly synthesized 9-arylimino noscapinoids, 1**2-14** was prepared with dimethyl sulfoxide (DMSO) and stored at 4 oC until use. The cells were allowed to grow at a temperature of 37 °C in a 5% CO2 and 95% humidity in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells with a 70-80 % confluence were sub cultured for bioassays using trypsin-EDTA (0.25 %).

***Cellular proliferation using MCF-7 and MDAMB-231 cell lines***

Antiproliferation activity of 9-arylimino noscapinoids, 1**2-14** was performed in 96-well plates as described previously using two human breast cancer cell lines, MCF-7 and MDAMB-231 [24]. In brief, cells were grown in DMEM culture medium supplemented with 10% FBS, 1% penicillin/streptomycin and 2 mM l-glutamine at 37 °C in a humidified atmosphere with 5% CO2. Cells were plated at a density of 5x103 cells per well and were treated with gradient concentrations (5 to 100 µM) of noscapine and its derivatives, 9-arylimino noscapinoids, 1**2-14** for 72h. The cells were then fixed with 50% trichloroacetic acid and stained with 0.4% sulforhodamine B. The unbound dye was removed by washing. The protein bound dye was then extracted with 10 mM Tris base and measured the optical density at 564 nm using a SPECTRAmax PLUS 384 microplate spectrophotometer. The IC50 value that stands for the drug concentration required to achieve a cell kill of 50% was determined using the online tool Quest GraphTM IC50 Calculator (AAT Bioquest, Inc., Sunnyvale, CA, USA, <https://www.aatbio.com/tools/ic50-calculator>).